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Úloha faktorů výživy v rozvoji diabetu 1. typu
Dietary factors in the development of type 1 diabetes

Bakalářská práce

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Tímto bych rád poděkoval svému školiteli MUDr. Davidovi Fundovi, Ph.D. za vedení mé bakalářské práce.

Prohlašuji, že jsem tuto bakalářskou práci vypracoval samostatně a že jsem řádně uvedl všechny použité prameny. Tato práce ani její podstatná část nebyla použita k získání jiného akademického titulu.

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Abstrakt

Diabetes mellitus 1. typu je autoimunitní onemocnění, které se vyvíjí u geneticky náchylných jedinců a jehož výskyt se zejména ve vyspělých zemích rychle zvyšuje. Rozvoj tohoto onemocnění je často spojován s faktory prostředí: viry, stresující životní události nebo absence expozice antigenům v raném životě zvyšují jeho výskyt v populaci. Antigeny, kterým jsme nepřetržitě vystavováni, jsou obsaženy v potravě. Lepek, mléčné bílkoviny nebo příjem prekurzorů vitaminu D jednoznačně ovlivňují patogenní proces diabetu mellitu 1. typu.

Tato bakalářská práce si klade za cíl popsat naše současné znalosti o úloze výživových faktorů u diabetu 1. typu, jejich možných imunitních mechanismech a interakcích s dalšími vnějšími faktory.

Klíčová slova: diabetes 1. typu, faktory výživy, bezlepková dieta, imunitní mechanismy, prevence, NOD myš, slizniční imunita

Abstract

Type 1 diabetes mellitus is an autoimmune disease which develops in genetically susceptible individuals and whose incidence rapidly increases, especially in developed countries. Type 1 diabetes is believed to be strongly associated with the environment: viruses, stressful life events or the absence of exposition to antigens in early life increase its incidence. Antigens to which we are expressed continuously are food antigens. Gluten, milk proteins or the intake of vitamin D precursors clearly influence type 1 diabetes pathogenic process.

This bachelor's thesis aims to describe our current knowledge on the role of dietary factors in type 1 diabetes, their possible immune mechanisms and also interplay with other environmental factors.

Key words: type 1 diabetes, dietary factors, gluten-free diet, immune mechanisms, prevention, NOD mouse, mucosal immunity

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1. Type 1 diabetes: An introduction

Type 1 diabetes/Insulin-Dependent Diabetes Mellitus (T1D/IDDM) is an autoimmune disease which leads to the destruction of pancreatic β-cells secreting insulin. Its incidence is around 0.4% within the population (Lefebvre et al., 2006; Scott, 1996).

However, the causes of the onset of T1D remain unclear, it is believed that besides genetic susceptibility the environmental factors such as viral infections, diet or vitamin D values play a critical role (Åkerblom et al., 2002) within the progress of this T-cell mediated disease from the preclinical form to the clinical condition as manifestations of the disease were reported in only 10% of genetically susceptible individuals (Knip et al., 2005; Lefebvre et al., 2006).

This thesis aims to summarise our current knowledge on the role of dietary factors in type 1 diabetes and their possible immune mechanisms. As genetics and environmental factors, in general, are considered to participate in the development of T1D with a great significance, they must not be omitted.

1.1. Genetics of T1D

Undoubtedly, T1D onset is linked to genetic susceptibility which is mediated by HLA class molecules I and II. These molecules are generally responsible for regulation of the immune system and presenting antigens to T-cells, and so they play an essential role in immune responses to both infections or in autoimmune diseases. HLA molecules are encoded by the extremely polymorphic HLA genes on chromosome 6. Over the past 40 years, many studies have focused on which HLA genes are associated with T1D susceptibility. It has been found that the risk of developing T1D does not come from one particular gene but that it is rather due to a combination of susceptible genes of DRB1, DQA1 and DQB1 alleles (i.e. haplotypes DRB1*0405-DQA1*0301-DQB1*0302 or DRB1*0401-DQA1*0301-DQB*0302 were considered most susceptible) and protective genes. Non-HLA genes for insulin and protein tyrosine phosphatase (which takes a crucial part in immune down-regulation) were also reported as susceptible-ones but with much smaller impact in the comparison to the HLA genes (Erlich et al., 2008; Noble and Erlich, 2012).

1.2. Nondietary environmental factors

As mentioned above, environmental factors are thought to be the triggers of the diabetic disease process. However, Kostraba et al. (1993) found that gender or birth do not play any role, Knip et al. (2005) described that for example migration of certain population groups

from an area with low incidence to an area with high incidence means an increase of the T1D incidence within the groups' members.

Viruses. The actual exogenous triggers which have been found to be diabetogenic were viruses such as Rubella virus (causing the congenital rubella syndrome) (Gale, 2008) or enteroviruses, respectively, the infections they cause.

Enteroviral infections are often accompanied only with mild (if any) manifestations (especially within the respiratory system), and the primary replication of the virus is located in the small intestine or lymphoid tissue from where the virus can spread to other tissues including the pancreas (Åkerblom et al., 2002). Seasonal variability of the onset rate is also associated with diabetogenic enteroviruses: an increased onset rate has been recorded between June and March peaking in autumn in some studies and was in correlation with high levels of antibodies against coxsackievirus B serotypes than in control groups (Gamble and Taylor, 1969; Gamble et al., 1969).

The fact that pancreatic endocrine cells of patients with newly diagnosed T1D contain a lot more immunoreactive IFN-α transcripts in comparison to the nondiabetic patients may be considered as a definitive proof of a diabetogenic effect of coxsackievirus of the B serotype (Åkerblom et al., 2002; Foulis et al., 1987; Huang et al., 1995).

Antenatal and Perinatal Factors. Besides the ancestor's diabetes when the predisposition of the offsprings for T1D may be much higher, the highest odds ratio was associated with the maternal-child blood group incompatibility. The hypothesis of the mechanisms consists in the induction of intolerance to β-cell autoantigens. Also, it comes as a surprise that *"maternal non-smoking was associated with an increased risk of developing childhood T1D"* (Dahlquist and Källén, 1992). High maternal age (>35 years) is considered to be another risk factor with a twofold excess as well as amniocentesis with a threefold higher risk or an excessive weight gain during pregnancy. Other factors such as proteinuria, hypertension or prescription of analgesics were mentioned as possibly risky ones but with lower significance; it has also been found that an increased risk is related to high birth weight (McKinney et al., 1997; Stene et al., 2001).

Hygiene Hypothesis. The increase in T1D incidence was registered together with one of the allergic diseases including childhood asthma. An explanation of this phenomenon offers the hygiene hypothesis: it says that it is due to the low contact with specific infectious agents at the beginning of life which has a protective effect mediated by regulatory T cells. This is also supported with the fact there was a decrease of T1D incidence among children with a significant number of child contacts and exposure to infections with no correspondence between the risk and the time of exposure during their first year of life. A significantly higher risk for Caesarean delivery was reported (Devendra, 2004; Gale, 2002; McKinney et al., 2000).

Stressful life events. Stressful life events are able to transform the subclinical form of T1D to the clinical one by increasing the requirements of insulin. This is believed to be mediated by hormones such as cortisol and catecholamines, and the counteracts between them (Akerblom and Knip, 1998).

Also, other factors such as vitamin D values or dietary proteins represent a great significance in developing T1D and will be discussed further in this thesis. Some of the most important triggers of T1D summarises Figure 1.

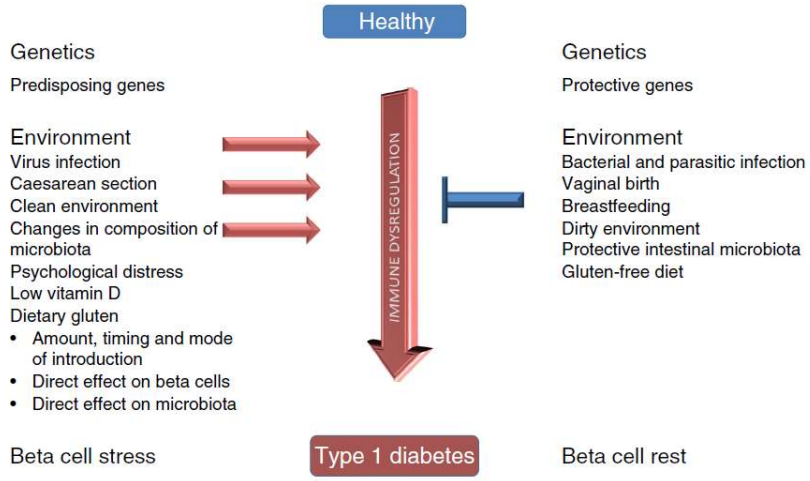

Figure 1 *shows that T1D development is conditioned by the interplay between genetic susceptibility and several different environmental factors.*

2. T1D and coeliac disease

Coeliac disease is an autoimmune gluten-sensitive inflammatory enteropathy in whose development genetic predisposition and environmental factors are involved as well. The disease manifests with gastrointestinal as well as nongastrointestinal symptoms, some patients (especially those with T1D) deny any symptoms. The diagnosis is based (besides biopsy of small intestine) on the levels of IgA AGA (antigliadin autoantibodies), tTG (tissue transglutaminase) as a putative indicator of AEA (anti-endomysial antibody) positivity or IgA ARA (anti-reticulin autoantibodies) (Dieterich et al., 1998; Green and Jabri, 2003; Holmes, 2001).

The coeliac disease occurs in T1D patients with a higher prevalence (4.4-11.1%) than in general population (0.5%) (Camarca et al., 2012). Other studies showed that autoimmune disorders such as T1D, autoimmune thyroid disease or connective tissue disease are a lot more frequent in patients with coeliac disease as well as in their first-degree relatives in general (Collin et al., 1994; Cooper et al., 1978; Ventura et al., 1999) and the coeliac disease offers several parallels with T1D.

Firstly, for both T1D and coeliac disease the antigen is believed to be commonly exposed to the population in developed countries (Knip et al., 2005) which might testify for a T1D dietary trigger. Secondly, the genetic predisposition for both diseases overlaps as the susceptibility is associated with partly the same HLA molecules DR3-DQ2 in the DQ region for both type 1 diabetes and coeliac disease, specifically DQB1*0302-DQA1*03 and DQB1*02-DQAl*05 alleles (either in *cis* or *trans*), with a contribution of TNF-α, a non-HLA gene (Camarca et al., 2012; Sumnik et al., 2006). This fact raises the question of whether endocrinologists should indicate an examination for the coeliac disease of patients with T1D automatically. An undiagnosed coeliac disease in insulin-dependent diabetes mellitus patients may lead to osteoporosis, infertility, T cell or B cell lymphoma or neurological and psychiatric disorders all of them worsening the quality of life or even increasing mortality (Green and Jabri, 2003; Holmes, 1996, 2001; Holmes et al., 1989).

Also, it was discussed in some studies whether dietary factors, specifically a gluten-free diet (GFD), may generally influence the severity of autoimmune diseases in patients with coeliac disease. However, some studies showed a significantly protective effect of GFD after a certain time of strict adherence to the diet (Hansen et al., 2006b; Lauret and Rodrigo, 2013), other studies refute these observations (Sategna Guidetti, 2001; Viljamaa et al., 2005). Ventura et al. (1999) also found a clear correlation between the risk of developing an autoimmune disorder and the age of diagnosis of a coeliac disease which testifies for the fact that duration of exposure of gluten plays an important role within the process.

3. Dietary influences and T1D

The observations mentioned above have led to the classification of external forces as uncompromising triggers of T1D, and nutritional factors have shown to be one of the most important ones (Hoorfar et al., 1991; MacLaren et al., 1989).

The principle of the research consists in feeding the research objects with nutrients of different composition: such differences between several test diets summarises Table 1.

Table 1: Composition of several test diets used in rodent model research (Hoorfar et al., 1991)

	Test diets (g/100 g)							
Ingredient	**Casein control**	**Hydrolyzed casein**	**Soybean meal**	**Wheat germ**	**Alfalfa seeds**	**Brewer's yeast**	**Red lentils**	**Plant protein mixture**
Protein source	0	20	35.5	40	60	10	42	36.16
Casein	20	0	0	10	0	15.5	8	10
Corn starch	65	52.95	41.1	27.9	19.6	55	17.38	20.97
Sucrose	0	12	12	12	12	12	24	24
Corn oil	5	5	4.3	2.76	2.84	5	4.58	4
Cellulose fiber	5	5	4	4.04	0.26	0	0	2.07
AIN-76 minerals	3.5	3.5	1.2	1.5	3.5	1	2.24	1
AIN-76A vitamins	1	1	1	1	1	1	1	1
DL-methionine	0.3	0.3	0.6	0.6	0.6	0.3	0.6	0.6
Choline bitartrate	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
L-tryptophan	0	0.05	0	0	0	0	0	0
Cysteine	0	0	0.1	0	0	0	0	0

3.1. Animal models

As it is impossible to carry out research on humans due to ethical limits, there are two types of rodent models used for investigating (not only) the dietary effects on T1D development: the non-obese diabetic (NOD) mouse and the BioBreeding diabetes-prone (BB dp) rat. These models are nowadays vital for the T1D research, and with their specific features, they bring a considerable amount of advantages.

The NOD mouse model is susceptible to T1D due to the same modification of MHC class II I-Aβ chain encoded by the DQ*0302 allele as humans which leads to the spontaneous development of diabetes. However, the insulin gene is responsible for T1D increased susceptibility; this is not with ease to determine within the NOD mouse model since it *contains two unlinked insulin genes, both of which are expressed*. Also, insulitis onset in humans is far from similarity in comparison to the one within the mouse model. In NOD mouse both $CD4^+$ and $CD8^+$ T cells participate in the autoimmunological process (leading to the destruction of β-cells by both necrosis and apoptosis) with defects antigen-presenting cells derived of bone-marrow, B lymphocytes, dendritic cells or macrophages all of which are considered

fundamental in autoimmunity. In BB rats, the process starts with T cell lymphopenia characterised by $CD4^+$ T cells reduction and absence of $CD8^+$ and regulatory T cells which are vital for impeding the immune system from a shift to autoimmunity. Afterwards, autoantibodies to islet cells, insulin or GAD can be detected. The BB rat takes advantage of lower maintenance costs and a better-defined genome. The BB rat spontaneously develops T1D as well (Atkinson and Leiter, 1999; Delovitch and Singh, 1997; MacMurray, 2002).

Both NOD mouse and BB rat are influenced by the environment significantly (Lefebvre et al., 2006).

3.2. Wheat proteins

Majority of cereal proteins component consists of proteins called prolamins. These endosperm storage proteins are of a higher molecular mass and are high in proline and glutamine. Two forms of prolamin proteins known as glutenins and gliadins form gluten. One of the reasons for gluten having a diabetes-promoting effect lies in its improper enzymatic processing in the intestine by gastric and pancreatic peptidase as well as the brush-border peptidase due to its prolin- and glutamine-rich character leading to an intestinal accumulation of gliadin peptides (Antvorskov et al., 2014; Piper, 2004; Shewry et al., 2002).

Within the following paragraphs, I will introduce several studies' findings.

It has been shown that diets which were based on cereals were highly diabetogenic whereas foods with a higher composition of hydrolysed or non-diabetogenic proteins were protective – the mice fed with wheat-based diet had notably higher amounts of transcripts of mRNA of T_H1-type and markers of IFN-γ, TNF-α or inducible NO synthase mRNA (all of them considered as proinflammatory) in comparison to the animals fed with diet of high composition of milk-protein fraction. This has been partly shown also in patients with coeliac disease (gliadin stimulates the production of inflammatory cytokines of T_H1 profile) and led to the suggestion that mucosal immune system could be partly responsible for the diabetogenic effect of diet. The expression of contrary cytokines IL-10 and TGF-β were unaffected. Also, T_H1/T_H2 ratio, one of the indicators of an autoimmune process, was eminently increased (Flohé et al., 2003; Jelínková et al., 2004).

Another study (Klemetti et al., 1998) has shown an increased cell-mediated immune response against gluten within newly diagnosed IDDM patients. Increased levels of IgA- and IgG-AGA have been reported but with no correlation to the proliferative response to gluten in IDDM patients. Both patients and controls were inside the reference limits of IgA-AEA. Thus, this study suggests that T-cell mediated immune response does not play an essential role in human IDDM pathogenesis.

Other studies have focused on the impact of gluten-free diet or, vice versa, gluten-enriched diet:

It has been shown by Funda et al. (1999) that the T1D incidence is noticeably decreased among NOD mice which were never exposed to gluten. The delayed T1D onset was reported as well. However, the number of $CD3^+$, TCR-$\gamma\delta^+$ and both Ig-A- or Ig-M-secreting cells were within limits in the proximal jejunum, and ileum and distal ileum and no significant differences across the dietary groups were observed, the gluten-free diet was suggested to serve as prevention of diabetes in NOD mice. Also, an association between T1D and coeliac disease was suggested.

Another study carried out on children with T1D-associated autoantibodies failed to observe the effects of a gluten-free diet on the basis of the fact that gluten antibodies levels and islet autoantibodies levels were not in correlation; however, in two subjects, the re-exposure to gluten led to increased levels of glutamic acid decarboxylase antibodies (GADA) and islet autoantibodies (IA-2A) and development of diabetes. Same observations were reported in other 3 cases – 3, 12 and 15 months after the gluten re-exposure. Overall, the increased levels of antibodies were observed only in 7 out of 29 cases; all the other instances were evaluated either with decreased or the same antibody levels (Hummel et al., 2002).

In another study by Funda et al. (2008), it has been suggested that not just a gluten-free diet, but also gluten-enriched diet has a protective effect in IDDM development. A non-purified diet enriched with gluten was prepared with the intention of increasing T1D incidence; however, the result was precisely the opposite: no significant changes within $CD3^+$ and TCR-$\gamma\delta^+$ subsets and both Ig-A- or Ig-M-secreting mucosal lymphocytes were observed, and so it was suggested that high doses of gluten may lead to tolerance of the immune system. This phenomenon has already been described in a different study (Labeta et al., 1993). Another explanation of decreased T1D incidence might lie in the composition of gut microflora which has an essential impact on mucosal immunoregulation (Funda et al., 2008).

Another study shows a gliadin-caused immunological response observed after rectal gliadin instillation leading to an increased density of $CD25^+$ cells not only in the patients themselves but also in their siblings. This observation supports the idea of the presence of an abnormal mucosal immune response to gluten in a subset of patients with IDDM (Troncone et al., 2003).

As mentioned above, it is clear there are several different opinions on whether gluten does or does not influence the titers of autoantibodies associated with T1D or the development of T1D itself, respectively, yet substantial evidence for both premises is missing at the moment.

3.3. Milk proteins

There are five main proteins in cow's milk: caseins (70–80%), β-lactoglobulin (β-LG; 10%), α-lactalbumin (5%), γ-globulin (2%), and bovine serum albumin (BSA; 1%). The composition of cow's milk, however, differs from that of human milk in a total content of proteins which is higher in cow's milk because of the higher concentration of casein and β-LG which is not present in human milk at all (Harrison and Honeyman, 1999).

The hypothesis of milk having an impact on the development of T1D was first expressed in 1980's. It has been shown that T1D incidence and cow's milk consumption or neonatal feeding practices are related, however, it differs among populations. I.e., the incidence of T1D in Finland and Sardinia is comparable (30/100,000 per year), but the milk consumption in Sardinia is half of the consumption in Finland. This is believed to be due to a different genetic predisposition to increased immune response to milk proteins associated with the HLA haplotype A1-B8-DR3-DQ2 (A1*0501, B1*0201) which also predisposes celiac disease (Fava et al., 1994; Gerstein, 1994; Harrison and Honeyman, 1999; Scott et al., 1996).

Another evidence of milk proteins having an impact on T1D development has been observed: BBDP rats fed with highly diabetogenic Purina Chow (PC) diet developed insulitis in 100% and 76% developed diabetes in less than 120 days. On the other hand, only 27% of rats fed with diabetes-retardant hydrolysed-casein (HC) diet developed only an early-stage insulitis and 18% developed diabetes. A hyper-expression of MHC I molecules of β-cells of each animal fed with diabetogenic PC diet was noted. However, MHC II molecules were not hyper-expressed notwithstanding the diet. The hyper-expression was in correlation with subsequent insulitis, diabetes respectively, and was not detected in any other tissue than pancreas. Also, the diabetes incidence was significantly reduced after monoclonal antibodies against MHC I molecules, which prevent $CD8^+$ T-cells from recognising pancreatic β-cells, were administered to NOD mice treated and untreated with cyclophosphamide. It is believed that the cause of induction of MHC I molecule hyper-expression needs to be found in a specific component of PC diet. (Li et al., 1995; Taki et al., 1991).

As milk is a significant nutrition source in the neonatal period, it has also been discussed whether the duration or even absence of breastfeeding has an impact on the T1D development. Mayer et al. (1988) and Nigro et al. (1985) showed a protective effect of breastfeeding but without significant odds ratios, Kostraba et al. (1993) and Virtanen et al. (1992) showed an opposite phenomenon but with nonsignificant odds ratios as well and suggest that *a history of cow's milk exposure before 3-4 months of age may be more relevant for type I diabetes than the total duration of exposure as no consistent dose-response relationship was apparent before 3 months of age.* Ziegler (2003) observed that the islet autoantibody risk was lowest among never breastfed children and highest among children breastfed six

months or more and suggests that early exposure to food supplements might be even beneficial in some individuals.

When it comes to possible mechanisms mediated by *humoral immunity,* it is generally accepted that antibodies to BSA are not responsible for the T1D development as there were only slight differences between individuals with recent-onset and the control groups (Atkinson et al., 1993; Ivarsson et al., 1995).

Also, increased levels of IgA to whole cow's milk proteins and bovine β-LG, as well as increased levels of IgG to β-LG in individuals with T1D onset, have been reported. The phenomenon of increased levels of IgG was seen primarily in children of the age of fewer than three years. Increased levels of IgA to cow's milk proteins were also reported in nondiabetic siblings (Savilahti et al., 1993). On the other hand, IgG antibodies to cow's milk proteins are considered physiological because they are recorded practically in all infants exposed to cow's milk (Harrison and Honeyman, 1999).

Another study (Saukkonen et al., 1998) reported higher levels of IgG to BSA and β-LG in children with T1D in comparison to their nondiabetic siblings with whom they shared the HLA-DQB1 susceptible alleles for IDDM which are linked to the stronger immune reaction.

A mechanism which is supposed to mediate the *cellular immune* reaction to cow's milk proteins is a phenomenon called *molecular mimicry* which consists in a cross-reactivity between cow's milk proteins and islet autoantigens.

For example, over 90 % of diabetic patients showed a tremendous proliferative response to BSA as well as to the ABBOS peptide whereas control cases did not. The ABBOS peptide is a 17 amino-acids albumin peptide which is considered as a reactive epitope due to their partly homologous sequence and against which antibodies react with the p69 β-cell surface protein (Cheung et al., 1994; Karjalainen et al., 1992).

Vaarala et al. (1996) found a significant immune response to BLG, however, didn't find an enhanced immune reaction to any other cow's milk proteins, i.e. immune response to BSA was low in all diabetic patients. Atkinson et al. (1993) have observed same results. The enhanced immune reaction was not related to genetic susceptibility.

Another study (Cavallo et al., 1996) has found that 51.1% of IDDM patients had an increased immune response to β-casein. In contrast, this had not been seen in healthy controls (2.7%) or patients with another disease with an autoimmune character, a thyroid disease (0%). Also, no correlation between response to β-casein and blood glucose, C-peptide, genetic susceptibility or age has been found. Ellis et al. (1998) has made a significant discovery when seen that not only diabetes patients but also their nondiabetic relatives demonstrated a high immune response against β-casein whereas healthy controls did not. This is believed to be associated with the similarity of HLA-DR types within the diabetic patients and their relatives.

As mentioned above, it is generally accepted that neonatal exposure to cow milk proteins does influence the T1D development in genetically susceptible individuals, however, what the character of the influence of breastfeeding is contradictory. On the other hand, the studies showing these observations lack appropriate controls, do not show a causal connection or usually focus only on a short period of life. Also, the HLA matching is the utmost necessity, and so more studies must be required (Harrison and Honeyman, 1999).

3.4. Vitamin D

Saggese et al. (1989) observed that, in vitro, vitamin D reduces proliferation activity, and, thus, production of cytokines. Hyppönen et al. (2001) perceived negative correlation between the intake of vitamin D and the incidence of T1D especially during the first year of life and suggests that vitamin D inhibits the autoimmune reaction towards pancreatic β-cells. Another study (Mathieu et al., 1992) found that a long-term vitamin D administration leads to a significant decrease of the occurrence of insulitis in NOD mice: 75% of controls developed diabetes whereas only 42% of mice treated with vitamin D showed signs of insulitis. Vitamin D is a stimulator for β-cells by which they produce higher amounts of insulin. Also, the advantage of the immunosuppression by vitamin D consists in its short-term effect as the MLR of splenocytes of the vitamin D-treated mice were normal in 24 hours. This is also why vitamin D should be considered as a potential medicament in the future. These findings have also been confirmed by other studies (Fournier et al., 1990; Lemire and Archer, 1991).

Due to Mathieu et al. (1994), even though vitamin D is considered to be a potent inhibitor of proliferation of T cells, significant immunosuppression has not been observed since splenocytes from both groups proliferated normally in MLR. Moreover, the splenocytes' co-transfer from vitamin D treated animals to naïve NOD mice delayed the onset of T1D significantly.

Vitamin D also stimulates monocyte differentiation and enhances suppressor function. Unfortunately, vitamin D plays a vital role in the metabolism of calcium, has a robust calcaemic effect, and so cannot be used as a prevention of T1D by itself in the long term. This is why synthetic analogues with a decreased calcaemic impact could surmount this problem in the future (Manolagas et al., 1990).

Similar effects have already been observed in studies of Endres et al. (1989) and Hughes and Pinder (2000) where cod liver has been administered to pregnant women to increase their vitamin D intake. The studies found a strong negative correlation between the IDDM incidence of the new-born infants and mother taking cod liver during pregnancy. This was attributed to the fact that long-chain n-3 fatty acids are linked to the decrease of IL-1 and

TNF production by mononuclear cells, and also the expression of HLA class II molecules on activated human monocytes and ICAM-1.

4. Immune mechanisms and possible modifications of T1D

There are dozens of hypotheses describing possible mechanisms through which this disease develops. Due to the scope of this work, I will select only some of them.

4.1. Leaky gut

An increased gut permeability of certain substances has been observed in IDDM patients (Carratù et al., 1999; Mooradian et al., 1986) and further suggested as a potential mechanism by which some antigens such as viruses, bacterias or dietary antigens could, in the end, trigger the immune response against β-cells. The abnormal changes are thought to be associated with claudins and occludins, vital proteins of tight junctions, whose abundance was significantly decreased in BBDP rats (Lüllmann-Rauch, 2012; Neu et al., 2005). Due to other studies, the pathway was suggested to be paracellular (Balda et al., 1996; Hollander, 1999).

4.2. Activation of the gut immune system

Glutamate decarboxylase of 65 kDa and 67 kDa molecular weight (GAD65/67) is expressed by cells of several different tissues, including pancreas, and is considered a key autoantigen in the development of IDDM as it is a target for both cellular and humoral immunity (Harrison et al., 1993; Kaufman et al., 1993).

The hypothesis of gut immune system activation lies in the fact that a specific population of lymphocytes, which also react with GAD65/67, expresses α4β7-integrin on its surface by which the lymphocytes recirculate to the gut lymphoid tissue, Peyer's patches, where their activity might be enhanced by dietary diabetogens. This recirculation is mediated by the MAdCAM-1 adhesion molecules in islet-close vessels, a ligand for a gut-homing receptor which is expressed in the islets at an early stage of insulitis. The T cell reaction against GAD65/67 is detectable in early stages of insulitis and is restricted only to lymphocytes expressing α4β7-integrin, however, after long-lasting insulitis, this phenomenon was not seen suggesting that other autoimmune-reactive, other than α4β7-, lymphocytes were present. As a proof, depletion of peripheral blood lymphocytes leads to a significantly decreased response of cellular immunity against GAD65/67 at early stages as well as treating neonatal NOD mice with monoclonal antibodies due to 1) generalized decrease in immune response; 2) blocking adhesion events leading to migration to pancreas, or 3) the change in T_H1/T_H2 ratio. (Paronen et al., 1997; Tisch et al., 1993; Yang et al., 1997).

The gut immune activation also consists in the higher presence of IL-1- and IL-4-positive cells. IL-4 is part of a T_H2 mediated response, and its production of gut-derived immune

cells leads to the increase of permeability of the small intestine as well as the antigen uptake and presentation by macrophages. A higher expression of ICAM-1 was confirmed (Carol et al., 1998; Falcone et al., 2001; Kodelja et al., 1998; Westerholm-Ormio et al., 2003).

4.3. The phenomenon of venular leakiness

A venular leakiness in pancreata has been observed in mice treated with the Monastral Blue with a suggestion that a phagocytosis of this colour by intravascular monocytes and their subsequent activation induces an increase in production of agents increasing permeability such as serotonin, leukotriene E4, histamine or bradykinin, probably only when it comes to animals susceptible to venular leakage. (Adams and Hamilton, 1984; Majno et al., 1987)

Inflammatory agents such as interleukin-1 (IL-1), gamma-interferon (IFN-γ), tumour necrosis factor (TNF), or lipopolysaccharides (LPS) cause endothelium more adhesive which leads to the trapping of monocytes. (Bevilacqua et al., 1985; Duijvestijn and Hamann, 1989; Joris et al., 1987; Majno et al., 1987)

4.4. Eosinophils as a source of α-defensin molecules

Increased levels of mRNA transcripts of α-defensin in the capillary in comparison to venous blood by a particular subpopulation of granulocytes have been observed among 30% of patients with T1D. These granulocytes were later identified as eosinophils, an essential element of innate immunity. The difference between venous and capillary blood defensin expression was suggested to be due to eosinophils' enhanced adhesion ability to structures of peripheral vessels. Since α-defensin molecules are considered to be associated with specific autoimmune and inflammatory diseases, it has been suggested that they might play some role, besides the always outspoken T-cells, in T1D development as well (Ganz, 2003; Håkansson et al., 1995; Neuwirth et al., 2012).

Data showing α-defensin is also a part of atherosclerosis (Nassar et al., 2007) or systemic lupus erythematosus (Sthoeger et al., 2009) suggest it could be used as a preclinical marker for chronic low-grade inflammatory disease in general (Neuwirth et al., 2012).

4.5. Eotaxin and eosinophils

Another study (Hessner et al., 2004) suspects eosinophils from a share in diabetes development. In this study, the authors observed a 5-fold increase expression of eotaxin, a chemotactic attractant of eosinophils, and FcεRI, a high-affinity IgE receptor, in pancreatic lymph nodes of the DR lyp/lyp mutant model, which spontaneously develops diabetes, at an early stage of eosinophilic insulitis. The enhanced eotaxin lymph node expression was

not seen in the DR +/+ model or Wistar-Furth (WF) rats, in both of which diabetes does not develop spontaneously. Other expressed transcripts of eosinophils, mast cells and lymphocytes were observed. Because pancreatic islets exhibited positive staining for anti-eotaxin antibody for both the DR lyp/lyp and DR +/+ murine model, it was suggested that eotaxin is, besides leukocytes, produced by the β-cells themselves. This was not seen in the WF rats as well.

4.6. Macrophage infiltration and silica administration

It has been suggested that, besides T-lymphocytes, macrophages play an important role in the pathogenesis of T1D as well. Firstly, macrophages are thought to be attracted to β-cells autoantigens probably via over-expressed MHC I class molecules of some regions of islets which then leads to an infiltration of other immunocytes (in the order of T- and NK cells, later B-cells). *This area was afterwards infiltrated by MHC class II positive cells which were identified as W3/25^{+}-, EDl^{+}-macrophages whereas B-, T- or NK-lymphocytes were almost absent at this stage.* Infiltration of lymphocytes was then always associated with a very strong infiltration of macrophages, and it was suggested that macrophage infiltration precedes lymphocyte infiltration. In conclusion, CD4^{+}, MHC class II positive, ED1^{+}, ED2^{-} activated macrophages are the first cells to infiltrate pancreas and causing the early stage of insulitis (Daniel et al., 1995; Hanenberg et al., 1989; Lee et al., 1988).

However, macrophages are not considered as primary effector cells and do not play an essential role in the β-cell destruction itself, they are necessary for the acceleration and even the initiation of T1D development, presenting specific antigens to T-cells or secreting products, both of which lead to β-cell destruction. Moreover, it has been observed that an early short-term administration of silica has a preventive effect regarding T1D pathogenesis as silica has a cytotoxic effect on macrophages. On the other hand, the late short-term administration had no impact suggesting that the start time of the treatment affects the prevention (Allison, 1966; Hanenberg et al., 1989; Ihm et al., 1991).

Evidence macrophages are essential for developing diabetes has been brought by Hutchings et al. (1990). Treating NOD mice with a CR3 monoclonal antibody which prevents macrophages from infiltrating pancreatic islets significantly reduced diabetes incidence.

Table 2: List of monoclonal antibodies (Hanenberg et al., 1989)

Antibody	Specificity
ED1	Cytoplasmic vacuole antigen on some free & tissue macrophages
ED2	Membrane antigen on some tissue macrophages
W3/25	Rat CD4 antigen on helper T cells (thymocytes, macrophages)

4.7. L3T4⁺ T-lymphocytes

An L3T4 antigen of a specific subset of T_H-lymphocytes participates in, with the association of MHC class II molecules, antigen recognition. Treating NOD mice with antibodies against this L3T4 antigen had a positive effect on the treatment of T1D. Knowing the antibody inhibits most MHC class II-associated responses in mice, it has been suggested the autoimmune response is strongly associated with MHC class II molecules and T-lymphocytes. Moreover, a T_H-cell subset of an analogous antigen in humans should be considered in the future as a possible form of T1D treatment (Dialynas et al., 1983; Koike et al., 1987; Shizuru et al., 1988).

4.8. B-cell involvement in the T1D development

Treating NOD mice with anti-μ antibodies leads to the complete depletion of B220⁺ cells and prevention of diabetes. Stopping the antibody treatment leads to the reestablishment of insulitis and sialitis suggesting B cells might serve as antigen-presenting cells to CD4⁺ T-cells and potent enhancers of β-cell autoimmunity (see Fig. 2 and 3) (Epstein et al., 1994; Noorchashm et al., 1997).

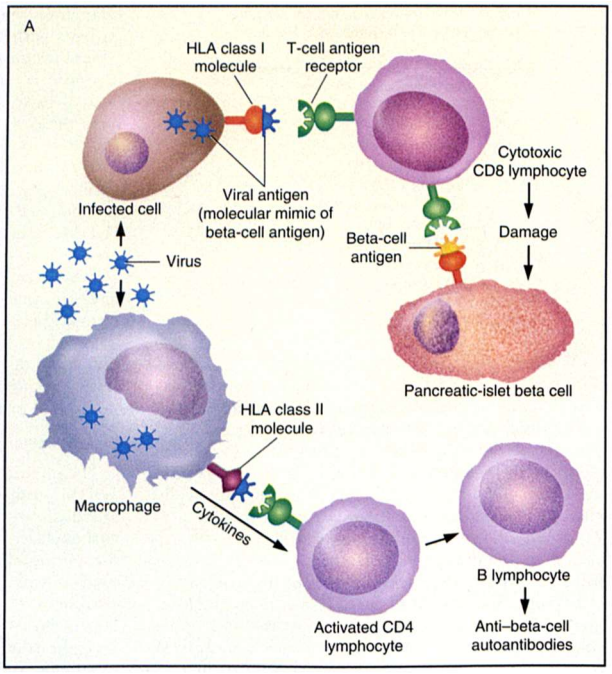

Figure 2: Interaction of components of the immune system. (Epstein et al., 1994)
*In the **molecular mimicry model**, a non-pancreatic cell is infected by a virus which leads to a presentation of antigens via HLA class I molecules, activation of cytotoxic CD8⁺ T-lymphocytes and β-cell damage. Moreover, anti-β-cells antibodies are secreted by B-cells previously activated by CD4⁺ T-cells on the basis of macrophages' HLA class II molecules.*

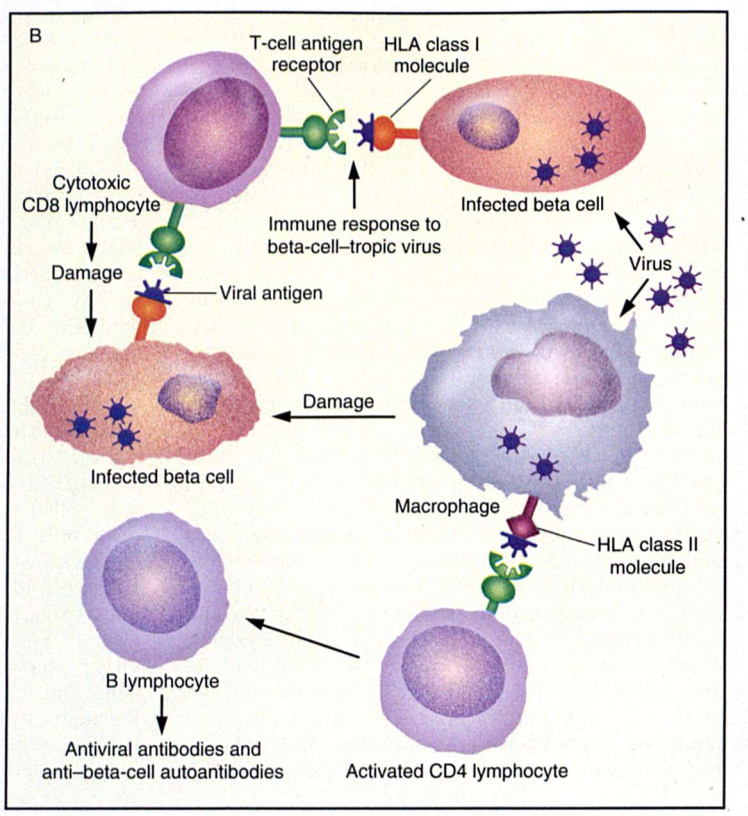

Figure 3: Interaction of components of the immune system. (Epstein et al., 1994)
Pancreatic β-cells themselves are infected by a virus, presenting antigens to T-cells, and later destroyed by CD8+ cytotoxic T-lymphocytes. Anti-β-cells antibodies are secreted by B-cells by the same mechanism.

4.9. (Pro)insulin peptides

Another possible tool for development of diabetes is the susceptibility of the immune system to the specific sequences of insulin or proinsulin, respectively.

The leading candidate sequence, to which over 93% of T cells are responsive, is the B:9-23 of insulin 2 B-chain. This peptide, when subcutaneously and without adjuvant administered to NOD mice, led, surprisingly, to the protection from T1D development. However, the mechanisms of the protection remain unknown. It has been observed that 1) the proliferative response to the B:9-23 peptide is significantly reduced in treated animals, 2) the production of cytokines of the T_H2 profile is increased (Daniel and Wegmann, 1996; Liu et al., 2003, 2006).

The findings mentioned above were later supported by an acceleration of diabetes with significantly increased insulin autoantibodies in mice with deficient expression of proinsulin 2. An enhanced immune response to 88-103 insulin A peptide was observed. (Thébault-Baumont et al., 2003).

Other studies have shown the protective effect of a monoclonal antibody against the peptide, YTS177.9. The prevention consists of targeting B:9-23-reactive T cells and suppressing

the humoral response of anti-B:9-23 and insulin autoantibodies. However, the administration of the mAb does not prevent insulitis (Hayward, 1993; Liu et al., 2003).

B:9-23-specific T_H1-like cells, producing contrary IL-4 and TNF at the same time, were observed to be the most diabetogenic among cell clones taken from NOD mice and administered to NOD/SCID mice, and so it was suggested that other cytokines are more important in the T1D development. Since B:9-23 specific T-cells occur from early stages of insulitis to the clinical form of diabetes, they might be a candidate target of intervention (Daniel et al., 1995; Hořejší, 2013).

(Chen et al., 2001) have found out that vaccination of NOD mice with proinsulin during perinatal period pronouncedly protects the animals from diabetes development. A homology of 13 amino acids between human proinsulin and GAD65 has been found (Rudy et al., 1995). Molecules of proinsulin are, apart from β-cells, expressed in thymus by a subset of follicular dendritic cells (Throsby et al., 1998) and then serve during the negative selection of T-lymphocytes. *Autoreactive T cells may be expanded in the periphery upon stimulation with a cross-reactive autoantigen(s) such as GAD65, ICA69, or IA-2, and result in a high frequency of (pro) insulin-specific T cells* (Chen et al., 2001).

Another possibility of diabetes prevention is a prediabetic administration of insulin in diabetes susceptible individuals since resting β-cells are less likely to be attacked by the immune system (Keller et al., 1993; Palmer et al., 1989).

5. Microbiota

It is known microbial colonisation begins as early as during birth delivery and that microbiota itself has an immense impact on the health of individuals later in life. Since it plays a crucial role in the education of the host immune system and the incidence of T1D among children born by Caesarean section was significantly higher, it is likely that microbiota plays a specific role also in the T1D development (Cardwell et al., 2008; Gensollen et al., 2016; Like et al., 1991).

The decline in Firmicutes and an increase in Bacteroidetes, as well as the decrease in functional diversity in metabolic pathways and stability, has been observed in autoimmune children. Unfortunately, many of the non-pathogenic strains of the healthy gut microbiome are poorly described as microbiology centres the pathologic ones. Also, the demands of autoimmune microbiome for specific nutrients were increased (Brown et al., 2011; Giongo et al., 2011; Vaarala et al., 2008).

The dysbiosis between Bacteroidetes and Firmicutes in other diseases such as type 2 diabetes (Larsen et al., 2010), Crohn's disease (Frank et al., 2007) or obesity (Turnbaugh et al., 2006) has been observed.

A high permeable intestine with inflammation has been seen in BP rats before T1D onset (Neu et al., 2005). However, it is unclear whether *the aberrant response to food or microbial antigen is the trigger of intestinal inflammation and increased permeability or vice versa* (Vaarala et al., 2008).

When it comes to dietary influences of the microbiome in T1D development, it has been observed that gluten-free diet significantly changes the microbial composition. The lower number of bacteria, especially the ones of gram-positive flora, has been found in the caecum. It is known that gluten-free diet protects mice from diabetes development and this might also be due to microbiota. Firstly, bacterias process lipopolysaccharides and convert them into short fatty acids such as acetate, propionate or butyrate, which are then absorbed by colon epithelium. These acids may also be found in blood, reducing glycemic response to oral glucose and providing β-cells with a rest. Secondly, knowing intestine mucosa is a vital for immune maturation, it has been suggested changes in microbial composition might have effect on mucosal dendritic cells and their promotion of T_H1 or T_H2 response (Brighenti et al., 1995; Hansen et al., 2006a; Vaarala et al., 2008; Venter et al., 1990).

More evidence of microbiota having an impact on T1D development have been found (Calcinaro et al., 2005; Matsuzaki et al., 1997; Yadav et al., 2007).

Finally, it has been suggested that the stability and the difference in ratio between Firmicutes and Bacteroides could be used as an early diagnostic marker (Giongo et al., 2011).

6. Conclusion

The type 1 diabetes pathogenesis is a very complicated process which is difficult to understand fully. Considerable progress has been made in defining immunologic markers, i.e. autoantibodies to insulin, glutamic acid decarboxylase or islet antigens, which are to predict the onset of the disease as they are detectable early in the asymptomatic phase. However, it remains unclear what are the actual triggers and mechanisms by which they operate (Atkinson and Eisenbarth, 2001; Neu et al., 2005).

When it comes to diet, it has been observed wheat or milk proteins have a diabetogenic effect. However, some studies have reported adverse observations. Proinflammatory cytokines were generally produced based on gluten-containing diet offering us a similarity with coeliac disease. The T_H1/T_H2 ratio has also been markedly increased as well as some autoantibodies.

Both cellular and humoral immune response have been observed in association with a diet containing milk proteins, especially β-LG, BSA and β-casein. Whether breastfeeding has a protective or retardant effect has not yet been fully elucidated.

On the other hand, vitamin D has a diabetes-retardant effect. It has an immunosuppressive effect, and so could be used as a preventive substance in high-incidence areas and susceptible individuals or their relatives. Further research should focus on the vitamin D synthetic analogues which would lack such a calcaemic effect.

Several mechanisms were proposed to participate in T1D development. An increased permeability, leakiness of pancreatic venules or recirculation of specific lymphocytes based on superficial antigens have been observed. Several autoantigens of β-cells such as GAD65, IA-2 or insulin have been found. Both innate and acquired immune system participate in the development.

As it is a disease of a continuously increasing incidence, many scientists have focused and should focus on its diagnosis and treatment. There are two main groups of treatment possibilities: 1) suppressing T-cell function, or 2) modulating the immune communication, among which may belong administering autoantigens to β-cells via nasal or oral route, modulating T_H1 to T_H2 pathway through expression of costimulatory molecules or using specific cytokines, their receptors or receptor antagonists leading to the decrease of production of β-cell destructive cytokines of a inflammatory profile (Atkinson and Leiter, 1999; Rabinovitch and Skyler, 1998).

T1D usually develops in young adults which are then sentenced to life-long, usually not very effective, treatment. They suffer from comorbidities and complications. As the incidence increases strikingly, it is necessary to find the actual triggers of the disease and describe the mechanisms by which they cause. Then, it will be easier to find a treatment

with proper effects as well as to design an efficient preventive plan for genetically susceptible individuals (Atkinson and Eisenbarth, 2001; Inzucchi and Sherwin, 2012).

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